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Phytochemistry 67 (2006) 191–201
PHYTOCHEMISTRY
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Flavonols and an indole alkaloid skeleton bearing identical acylated glycosidic groups from yellow petals of *Papaver nudicaule*

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Received 27 September 2005; received in revised form 27 October 2005

In memory of Martin Luckner

Abstract

From yellow petals of Iceland poppy, besides the known flavonoid gossypitrin, seven kaempferol derivatives were isolated. In addition to kaempferol 3-*O*-β-sophoroside and kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside, known from other plants, the mono- and dimalonyl conjugates of the latter were identified by MS and NMR spectroscopy. Structure analyses of a set of co-occurring pigments, the nudicaulins, revealed that they have the identical acylated glycoside moieties attached to a pentacyclic indole alkaloid skeleton for which the structure of 19-(4-hydroxyphenyl)-10*H*-1,10-ethenochromeno[2,3-*b*]indole-6,8,18-triol was deduced from MS and NMR as well as chemical and chiroptical methods.

Keywords: *Papaver nudicaule*; Papaveraceae; Iceland poppy; Flower pigments; Kaempferol glycosides; Malonyl conjugates; Nudicaulins

1. Introduction

Garden varieties of the Iceland poppy (*Papaver nudicaule* hort. non L.; Hanelt, 1970) show red, orange and dark yellow coloured flowers as well as the pale yellow ones typical of the native species *P. nudicaule* L. (Fabergé, 1942). White flowers occur in cultivated and wild populations. Whereas pelargonidin 3-*O*-β-sophoroside-7-*O*-β-glucoside (orientalin) (Harborne, 1963) and pelargonidin 3-*O*-β-[(6-malonyl)sophoroside] have been identified from the red variety (Cornuz et al., 1981), the pigments from other varieties have received scant attention. Thus, the major pigment from the dark yellow petals of *P. nudicaule* was named nudicaulin (Price et al., 1939), while a minor component was thought to be quercetagetin 7-*O*-β-glucoside (Harborne, 1965) but finally identified as gossypitrin (gossypetin 7-*O*-β-glucoside) (Harborne, 1969). Early investigations of the nudicaulin structure by elemental analysis, oxidative degradation and acid hydrolysis suggested the presence of nitrogen and a 4-hydroxyphenyl moiety in a diglucosidic compound (Price et al., 1939). Later a triglucoside was claimed from enzymic degradation and the spectral properties of the aglycone indicated that nudicaulin is neither a flavonoid nor a carotenoid or a betaxanthin (Harborne, 1965). In the present study we report on the analysis of the complex natural product pattern of yellow petals of *P. nudicaule* consisting of eight flavonoids and eight nudicaulins. Besides the known gossypitrin (Harborne, 1969), four kaempferol

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doi:10.1016/j.phytochem.2005.11.002

derivatives were fully characterised and the elucidation of nudicaulins containing an elusive aglycone structure is described.

2. Results and discussion

2.1. Flavonol glycosides

HPLC analysis of an extract from lyophilised petals of yellow flowering Iceland poppy (Fig. 1) revealed the presence of eight flavonoids (**1**–**8**) and eight nudicaulins (**I**–**VIII**) by detection at 350 and 460 nm, respectively (Fig. 2 and Table 1). The spectral data of seven flavonoids are nearly identical pointing to the presence of a group of closely related kaempferol derivatives (Harborne, 1988). After isolation by preparative HPLC, four flavonols were identified by MS, sugar component and linkage analysis and by NMR as kaempferol 3-*O*-β-sophoroside (**7**) and its derivatives: kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside (**1**), kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-glucoside (**2**) and kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-[(6- malonyl)glucoside] (**5**) (Fig. 3). For **2** the ^{1}H NMR data revealed the presence of one acyl group attached to the C-6 of the inner glucose of a sophorose moiety which was confirmed by detailed analysis of the positive ion ESI-MS fragmentation pattern of the $[M+H]^{+}$ and comparison with the analogous pattern of **5** whose structure was unambiguously determined from the NMR data (Table 2). Comparable ESI-MS data indicated that compounds **3**, **4** and **8** are structural isomers of **5**, **2** and **7**, respectively, but for lack of sufficient material, NMR analysis was not possible. Furthermore, compound **6** was fully characterised as gossypitrin on the basis of both MS and NMR data (Table 3) and comparison with literature data (Scoelly and Kapetanidis, 1993; Wind et al., 1998). All kaempferol derivatives are new for *P. nudicaule* and, to the best of our knowledge (Harborne et al., 1975; Harborne, 1988, 1994), kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-glucoside (**2**) and kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-[(6-malonyl)glucoside] (**5**) are new natural compounds. Kaempferol 3-*O*-β-sophoroside (**7**), named sophoraflavonoloside, was isolated first by Rabaté and Dussy (1938) from unripe fruits of the Japanese pagoda tree [*Sophora japonica* L., now reclassified as *Styphnolobium japonicum* (L.) Schott, (Fabaceae); Santamour and Riedel, 1997]. Kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside (**1**) was characterised to some extent both from petals of petunia [*Petunia* x *hybrida* L. (Solanaceae)] (Birkofer and Kaiser, 1962) and dried stigmata of saffron [*Crocus sativus* L. (Iridaceae)] (Straubinger et al., 1997) and is now fully characterised from yellow petals of *P. nudicaule*, a member of the Papaveraceae.

Fig. 1. Flowers of Iceland poppy (*Papaver nudicaule*) as collected for investigation.

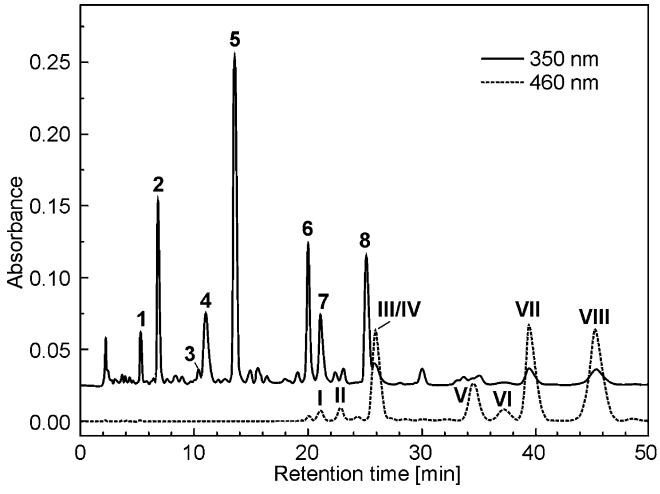


Fig. 2. HPLC elution profiles of pigments [PDA-detection: 350 nm (flavonoids); 460 nm (nudicaulins)] from yellow petals of *P. nudicaule*. Peak numbers (**1**–**8** for flavonols, **I**–**VIII** for nudicaulins) correspond to the numbers in tables and formula schemes. (Note: the absorbance at 350 nm was reduced (50%) to depict both chromatograms in one scheme).

2.2. Nudicaulins

The spectral properties of the kaempferol derivatives (**1**–**5**, **7**–**8**) indicate that these do not contribute to the flower colour and even gossypitrin (**6**) may only have a small impact. Hence the pigments responsible for the dark yellow petals are most likely the nudicaulins. The first substantial step after the initial studies (Price et al., 1939; Harborne, 1965) to disclose the nudicaulin structure was the separation of the pigment mixture by HPLC into eight components consisting of four pairs with similar chromatographic and spectral properties all of which show an absorption maximum at about 460 nm (Fig. 2 and Table 1, **I**–**VIII**). Solvent system 1, using 1% acetic acid as acidifying agent, afforded a baseline separation of the main

Table 1
Retention time, HPLC-PDA (solvent system 1), mp and MS data of flavonoids and nudicaulins from yellow petals of *P. nudicaule*

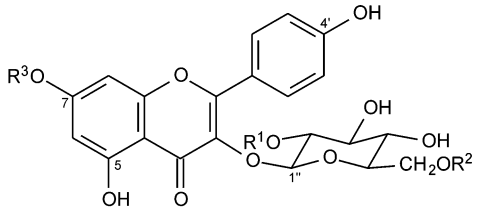
Compound	R_t (min)	HPLC-PDA λ_{max} (nm)	mp[a]°C	LCMS (*m/z*)			Structural assignment
				$[M+Na]^+$	$[M+H]^+$	$[M-H]^-$	
1	5.2	218/264/347	–	795	773	771	Kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside
2	6.8	215/264/347	184	881		857	Kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-glucoside
3	10.3	225/264/347	–	967	945	943	Isomer of **5**
4	10.9	226/264/346	–	881		857	Isomer of **2**
5	13.6	225/264/346	183	967	945	943	Kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-(6-malonyl)glucoside
6	20.0	215/259/270sh/340sh/384	203-205	503		479	Gossypitrin
7	21.1	222/264/347	189	633		609	Kaempferol 3-*O*-β-sophoroside
8	25.1	223/264/347	–	633		609	Isomer of **7**
I	21.0	225/254/338/460	–		872		Glc-A1-Glc-Glc
II	22.8	225/254/338/457	–		872		Glc-A1*-Glc-Glc
III	25.9	202/256/334/457[b]	–		958		Glc-A1*-(malonyl-Glc-Glc)
IV	25.9	201/256/334/460[b]	–		958		Glc-A1-(malonyl-Glc-Glc)
V	34.6	218/253/336/459	–		958		Isomer of **IV**
VI	37.3	221/253/336/455	–		958		Isomer of **III**
VII	39.5	222/255/335/457	–		1044		Malonyl-Glc-A1*-(malonyl-Glc-Glc)
VIII	45.4	222/255/335/460	–		1044		Malonyl-Glc-A1-(malonyl-Glc-Glc)
A	20.5[c]	222/256/334/458 [c]	–		710		A1-Glc-Glc
A*	20.9[c]	222/256/333/455[c]	–		710		A1*-Glc-Glc
Transformation product of **VIII (TP)**	9.9[d]	227/337[d]	–		1044		

Compound numbers correspond to peak numbers in Fig. 2.
[a] Uncorr.
[b] Spectral data (solvent system 3).
[c] R_t and spectral data (solvent system 4).
[d] R_t and spectral data (solvent system 2).

components **VII** and **VIII**, while **III** and **IV** eluted together. In contrast, with 1.5% phosphoric acid (solvent system 3) **III** and **IV** were well separated, but **VII** and **VIII** co-eluted (data not shown). The early suggestion that nudicaulin is a nitrogenous compound (Price et al., 1939) was supported by LC-MS of the main compound **VIII** which shows a protonated molecular ion at *m/z* 1044 (Hempel, J., Metzger, J.W., Böhm, H., 1998, unpublished). In subsequent high resolution mass spectrometric analysis of **VIII** using electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR-MS), the protonated molecular ion was found at *m/z* 1044.2593 ($C_{47}H_{50}NO_{26}$, calc. 1044.2616). Almost identical data were obtained for **VII** revealing that this is an isomer of **VIII**. By changing the capillary exit voltage an increased fragmentation was observed giving an informative pattern of fragments (Fig. 4) and pointed to the presence of two malonyl and three hexose moieties bound to an aglycone with an odd molecular mass of 385 (protonated aglycone ion *m/z* 386.1022, $C_{23}H_{16}NO_5$, calc. 386.1023).

Acid hydrolysis (2 N HCl, 30 min, 100 °C) converted**VIII** and **VII** to the isomeric compounds **A** and **A*** with different HPLC retention times and different spectral properties (see Table 1). HR-MS showed that this treatment caused the loss of two malonyl and one glucosyl moieties from both **VIII** and **VII** (Fig. 4). Treatment of all nudicaulins under these acidic conditions showed that the intermediates **A** and **A*** are present in all members and **A1** and **A1*** are the stereoisomeric aglycones in **I** to **VIII** (Table 1). Thus, **VIII** and **VII** are 6-malonylglucoside-A1-(6-malonylsophoroside) and 6-malonylglucoside-A1*-(6-malonylsophoroside), respectively. Following the kinetics of the alkaline hydrolysis of the dimalonyl conjugate **VII** by HPLC revealed the intermediary character of a monomalonyl compound that is identical with the endogenous component **III**. Further hydrolysis of this intermediate afforded a non-acylated final product which is identical with the endogenous compound **II**. From similar experiments and HR-MS data it is obvious that **VIII** and **VII** contain two malonyl groups and the other ones **III** to**VI** one malonyl moiety each, while **I** and **II** are their non-acylated precursors.

The UV/Vis spectra of **VII** and **VIII** in MeOH/H_2O are clearly different from spectra recorded in MeOH/H_2O containing 1% AcOH and have almost identical molar absorptivities in the latter (see Section 3.6.), with only a small shift (3 nm) of the highest λ_{max} between the two spectra. The specific optical rotation for **VII** is −354°, whereas the corresponding value for **VIII** is +370° suggesting an optical antipode. A similar conclusion was drawn from the CD-spectra under neutral conditions as **VIII** shows a positive molar ellipticity, while **VII** shows an inverted spectrum (Fig. 5); the small deviations are probably due to differences in purity.



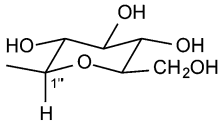
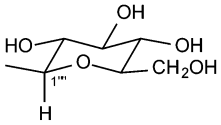
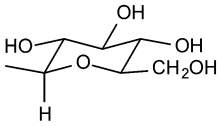
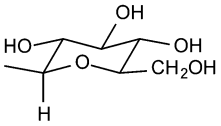
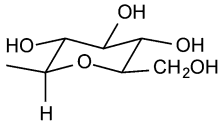
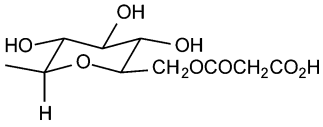
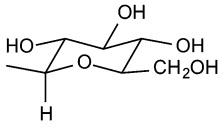
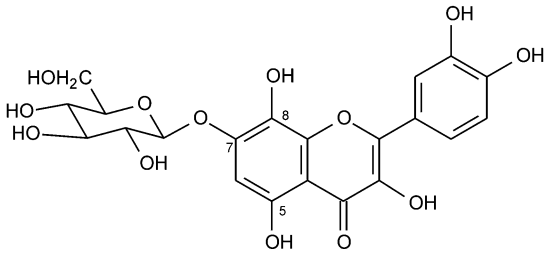
Flavonoid	R^1	R^2	R^3
1		H	
2		$-COCH_2CO_2H$	
5		$-COCH_2CO_2H$	
7		H	H
6			

Fig. 3. Structure scheme of flavonoids from yellow petals of *P. nudicaule*. (For the assignment of the NMR data, the inner and outer glucose of the sophorose moiety are designated as A and B, whereas the glucose attached at the C-7 is C. The malonyl residues at glucose A and C are consequently named A′ and C′).

An unusual reaction was observed during the preparative purification of **VII** and **VIII**. Analysis of the purified compounds by HPLC showed the presence (about 5%) of a compound with a different retention time and spectral properties (λ_{max} 227/337 nm) indicating a substantial alteration of the chromophore, but with the unchanged *m*/*z* of 1044 (Fig. 4). After isolation and concentration of this transformation product (**TP**), an almost complete reconversion to the starting material **VIII** and **VII** with the typical nudicaulin absorption maximum at about 460 nm was detected and corroborated by HR-MS (Table 1 and Fig. 4).

The ^{1}H NMR and ^{1}H–^{1}H COSY spectra of nudicaulins **VII** and **VIII** (Fig. 6) show three spin systems for the aglycones, which are described in detail in the following, using the data of the aglycone of **VIII**: (1) Two doublets ($J = 2$ Hz) of an AX system of H-5 (δ 6.12) and H-7 (δ 6.06) (ring **a**), resembling the protons of ring A of flavonoids, (2) two doublets of a characteristic AA′XX′ spin system of a *p*-substituted phenyl ring (ring **f**, δ 8.16, H-2′/6′ and δ 6.99, H-3′/5′) and (3) another AA′XX′ of a 1.2-disubstituted aryl ring (ring **d**, δ 7.97/7.50/7.38/7.21) (Table 4) belonging to the indole moiety.

^{1}H–^{1}H COSY, HSQC and HMBC correlations were used to assign all proton and carbon atoms within the corresponding substructures and established possible links to other parts of the molecule. For ring **a**, HMBC correlations of H-5 and H-7 with the quaternary C-9 (δ 103.5) indicated the latter as a bridgehead carbon for the link to C-10. Due to non-equivalent C/H-5/7 and C-4/8, a second connection to another part of the molecule was deduced via O-3. According to HMBC correlations with the protons of ring **d**, the quaternary C-11 (δ 136.4), C-12 (δ 124.6) and C-17

Table 2
^{1}H (500 MHz) and ^{13}C NMR data of kaempferol 3-*O*-β-[(6-*O*-malonyl)sophoroside]-7-*O*-β-[(6-*O*-malonyl)-glucoside] (**5**)

No.	DMSO-d_6		CD_3OD	
	δ, mult., J(Hz)	δ[a]	δ, mult., J (Hz)	δ[b]
Aglycone				
2		156.7		160.1
3		133.0		135.0
4		177.5		179.8
5		160.9		161.7
6	6.46, *d*, 2.1	99.4	6.50, *d*, 2.1	101.0
7		162.6		164.6
8	6.78, *d*, 2.1	94.5	6.75, *d*, 2.1	96.1
9		155.9		158.0
10		105.6		107.6
1′		120.6		122.7
2′/6′	8.04, *d*, 8.8	131.1	8.03, *d*, 8.8	132.5
3′/5′	6.92, *d*, 8.8	115.3	6.91, *d*, 8.8	116.3
4′		160.2		162.8
Glc A[c]				
1″	5.61, *d*, 6.3	98.1	5.37, *d*, 7.8	100.9
2″	3.50, *m*[e]	82.1	3.77, *dd*, 7.8, 8.8	82.1
3″	3.31[e]	76.1[#]	3.63, *dd*, 8.8, 7.7	77.6 [#]
4″	3.14–3.23[e]	69.3*	3.38–3.42[e]	71.1*
5″	3.34, *m*	74.0	3.39[e]	75.5
6″A	3.92, *dd*, 5.6, 10.6	63.4	4.09, *dd*, 5.2, 11.8	64.6
6″B	4.20, *d*, 10.6		4.25, *dd*, 11.8, 1.8	
mal-1″[d]		164.4		168.3
mal-2″A	3.01, *dd*, 15.9	41.0[$]	nd[f]	nd[g]
mal-2″B	3.06, *dd*, 15.9		nd[f]	
mal-3″		167.7[§]		170.1[§]
Glc B				
1‴	4.62, *d*, 7.8	104.1	4.76, *d*, 6.9	104.6
2‴	3.09, *m*	74.3	3.40, *m*[e]	74.7
3‴	3.14–3.23[e]	76.6[#]	3.53, *m*[e]	77.7 [#]
4‴	3.14–3.23[e]	69.7*	3.38–3.42[e]	71.5*
5‴	3.50[e]	76.3[#]	3.33[e]	78.3
6‴A	3.52, *dd*	60.8	3.69, *dd*, 5.2, 12.1	62.7
6‴B	3.50, *m*[e]		3.82, *dd*, 12.1, 2.0	
Glc C				
1⁗	5.11, *d*, 7.3	99.7	5.05, *d*, 7.4	101.5
2⁗	3.31, *dd*	73.0	3.51, *m*[e]	75.4
3⁗	3.14–3.23[e]	77.1	3.40, *m*[e]	77.9
4⁗	3.14–3.23[e]	69.5*	3.38 - 3.42[e]	71.3*
5⁗	3.75, *m*	73.8	3.79, *m*	75.6
6⁗A	4.13, *dd*, 4.8, 10.8	64.1	4.29, *dd*, 6.8, 11.8	65.4
6⁗B	4.40, *d*, 10.8		4.54, *dd*, 11.8, 1.8	
mal-1⁗		166.9		168.7
mal-2⁗A	3.37, *d*, 15.9	41.5[$]	nd[f]	nd[g]
mal-2⁗B	3.41, *d*, 15.9		nd[f]	
mal-3⁗		167.9[§]		170.2[§]

Exchangeable hydroxyl signals of carbohydrate moieties (DMSO-d_6): A: δ 5.54 (brs), 5.32 (*d*, $J = 6$ Hz); B: δ 5.48 (*d*, $J = 5$ Hz), 5.02 (brs), 4.94 (brs), 4.33 (*t*, $J = 5$ Hz); C: δ 5.49 (*d*, $J = 5$ Hz), 5.35 (*d*, $J = 6$ Hz), 5.26 (*d*, $J = 5$ Hz). Exchangeable carboxyl signals of the malonyl moieties (DMSO-d_6): δ 12.7 (br). Exchangeable phenolic signals of the aglycone (DMSO-d_6): δ 12.65 (*s*, 5-OH), 10.22 (*s*, 4′-OH).

*, §, #, $ may be reversed in one column.

[a] Chemical shift obtained from ^{13}C NMR spectrum at 125 MHz.

[b] Chemical shift obtained from ^{13}C NMR spectrum at 100 MHz.

[c] Glc, glucosyl.

[d] mal, malonyl.

[e] Chemical shift obtained from HSQC and HMBC spectra.

[f] Exchangeable protons, signal not detected.

[g] Reduced SN due to attached $^2H_2$, signal not detected.

Table 3
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of gossypitrin (**6**)

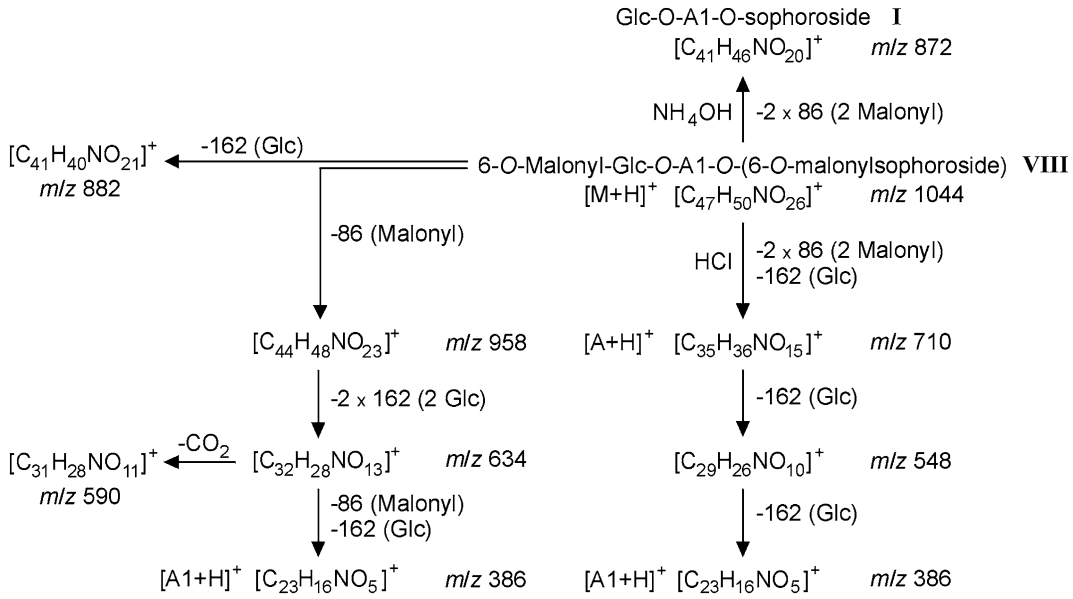
No.	DMSO-d_6		Me_2CO-d_6 (Wind et al., 1998)
	δ, mult., J(Hz)	δ[a]	δ (75 MHz)
2		147.3	148.2
3		135.6	136.9
4		175.9	177.3
5		151.0	151.2
6	6.61, *s*	97.8	98.9
7		150.0	152.4
8		126.6	128.2
9		143.1	144.4
10		104.4	105.7
1′		122.0	123.3
2′	7.75, *d*, 2.3	115.2	116.1
3′		144.7	146.1
4′		147.5	148.9
5′	6.89, *d*, 8.6	115.6	116.3
6′	7.62, *dd*, 8.6, 2.3	120.3	121.2
1″	4.91, *d*, 7.6	101.4	103.1
2″	3.34, *m*[b]	73.2	74.1
3″	3.41, *m*	75.3	78.3
4″	3.17, *m*	69.6	70.7
5″	3.29, *m*[b]	77.3	76.5
6″A	3.72, *dd*, 5.0, 11.0	60.6	61.7
6″B	3.47, *m*		

Exchangeable hydroxyl signals of carbohydrate moieties (DMSO-d_6): 2-OH: δ 5.40 (*d*, $J = 3.9$ Hz); 3-OH: δ5.13 (*br*, $J = 4.5$ Hz); 4-OH: δ 5.08 (*d*, $J = 5.3$ Hz): 6-OH: δ 4.64 (*t*, $J = 5.0$ Hz). Exchangeable phenolic signals of aglycone (DMSO-d_6): δ 11.92 (*s*, 5-OH), δ 9.62 (*s*, 4′-OH), δ 9.34 (*s*, 3′-OH), δ 8.56 (*s*, 8-OH).

[a] Chemical shift obtained from HSQC and HMBC spectra.

[b] Overlaid by HDO resonance.

(δ 162.5) were attributed to the indole substructure (ring **c**/**d**). The only nitrogen atom in the molecule (odd molecular weight m/z 1043, HR-MS) must be at attached to C-17, from its low-field shift. These data, together with the long-range HMBC correlation through four bonds of H-16 (δ 7.50) with the extreme low-field C-2 at δ 179.8, completed the assignment of the indole moiety. H-2′/6′ of the *p*-hydroxylated phenyl ring **f** exhibited a HMBC correlation with another quaternary low-field carbon, C-19 (δ 148.9), which is part of the link to the core structure. While there was no direct HMBC correlation between ring **a**, ring **c**/**d** (indole) and ring **f**, the additional methine at position 10 (δ 5.09/48.1) proved to be useful in establishing the connections between these substructures. H-10 exhibited HMBC correlations with seven quaternary carbons which, except for C-18 (δ 126.1), have already been identified as being part of ring **a** (δ 154.7, C-4; δ 159.3, C-8; δ 103.5, C-9), ring **c** (δ 179.8, C-2; δ 136.4, C-11), and the ring **f**-containing substituent (δ 148.9, C-19). An additional HMBC cross-peak of H-10 was observed with the carbon atom at δ 126.1, which therefore was attributed to C-18. Thus, C-10 forms the link between ring **a**, the nitrogen-containing ring **c** of the indole moiety, and the *p*-hydroxyphenyl vinyl residue. At this stage, the nature of rings **b** and **e** still have to be established. The unusual chemical low-field shift of C-2 (δ 179.8) cannot be explained only by its attachment to nitrogen but requires another electronegative substituent,



Ion	*m/z* found	*m/z* calculated	Elemental composition
[M+H]$^+$	1044.2593	1044.2616	$C_{47}H_{50}NO_{26}$
[(M+H)-mal]$^+$	958.2584	958.2612	$C_{44}H_{48}NO_{23}$
[(M+H)-mal-2Glc]$^+$	634.1552	634.1555	$C_{32}H_{28}NO_{13}$
[(M+H)-mal-2Glc-CO$_2$]$^+$	590.1652	590.1657	$C_{31}H_{28}NO_{11}$
[A1+H]$^+$	386.1022	386.1023	$C_{23}H_{16}NO_5$
[A+H]$^+$	710.2080	710.2080	$C_{35}H_{36}NO_{15}$
[(A+H)-Glc]$^+$	548.1539	548.1551	$C_{29}H_{26}NO_{10}$
[A1+H]$^+$	386.1022	386.1023	$C_{23}H_{16}NO_5$
[A*+H]$^+$	710.2081	710.2080	$C_{35}H_{36}NO_{15}$
[I+H]$^+$	872.2594	872.2608	$C_{41}H_{46}NO_{20}$
[V+H]$^+$	958.2588	958.2612	$C_{44}H_{48}NO_{23}$
[TP+H]$^+$	1044.2592	1044.2616	$C_{47}H_{50}NO_{26}$
[A+H]$^+$	710.2080	710.2080	$C_{35}H_{36}NO_{15}$

Fig. 4. Fragmentation pattern of **VIII** and its hydrolysis products in ESI-FT-ICR-MS and HR-MS data of **I** and **V** as well as of the transformation product of **VIII** (**TP**).

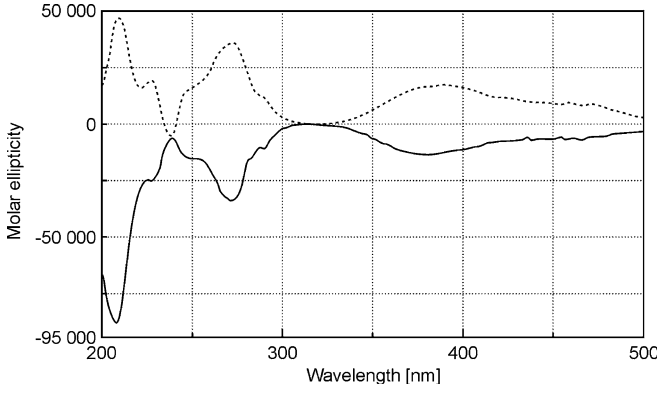


Fig. 5. CD-spectra (molar ellipticity $[\Theta]_M$) of **VII** (solid line) and **VIII** (dotted line).

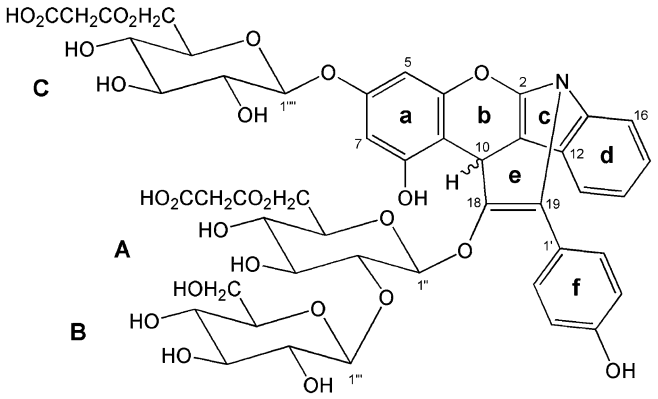


Fig. 6. Proposed structure of nudicaulins **VII** and **VIII** with opposite stereochemistry at the chiral C-10.

which is suggested to be the ether bridge oxygen atom in position 3, closing ring **b**. Likewise, the chemical shift of δ 148.9 is a strong argument for the existence of a bond between C-19 and the indole nitrogen, rather than between C-19 and the oxygen at C-8. Thus, the six-membered ring **e** containing the vinyl double bond is fused to ring **b** through C-2, C-11, and C-10, and has N-1, C-2, and C-11 in common with ring **c**. This structure is supported by an intense NOE between H-2′/6′ and H-13, indicating close proximity of **d** and **f**. A linkage of C-19 with O-8, instead of N-1, would result in an alternative ring including C-8 to C-10, C-18, C-19 and O-8. However, the chemical shifts of

C-18 (δ 126.1) and C-19 (δ 148.9) are inconsistent with this possibility. All data are compatible with a 19-(4-hydroxyphenyl)-10*H*-1,10-ethenochromeno[2,3-*b*]indole-6,8,18-triol as the aglycone of nudicaulins (Fig. 6).

Using ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, TOCSY, HSQC and HMBC experiments, identification of the sugar moieties was performed according to the strategy used for structure elucidation of compound **5**. Attachment of the carbohydrate units to O-6 and O-18 was established by means of HMBC correlations of the anomeric protons of glucose A (δ 4.59, H-1″) with C-18 and glucose C (δ 4.71, H-1‴″) with C-6 (δ 159.3). Another HMBC cross-signal between H-1‴ of glucose B (δ 4.38, H-1‴) and the downfield C-2″ (δ 81.8) of glucose A indicated the interglucosidic bond between the two glucose units A and B of the sophorose. The malonyl residues caused a low-field shift of the methylene carbon resonances of about 3 ppm to δ 63.9 (C-6″ and C-6‴″) and also affected the chemical shifts of protons H-6″A, H-6″B, H-6‴″A, H-6‴″B, H-5″ and H-5‴″ of the corresponding glucose A and C units (Table 4). In accordance with these results sugar methylation analysis revealed only glucose and sophorose. The linkages of the malonyl residues are corroborated by QTOF ESI-MS/MS of *m*/*z* 1044 giving a signal at *m*/*z* 882 $[(M+H)\text{-Glc}]^{+}$ pointing to an non-malonylated outer Glc residue.

Thus, we propose compound **VIII** is a 6-*O*-β-[(6-*O*-malonyl)glucoside]-18-*O*-β-[(6-*O*-malonyl)sophoroside] of 19-(4-hydroxyphenyl)-10*H*-1,10-ethenochromeno[2,3-*b*]-indole-6,8,18- triol, nudicaulin **VIII**. The diastereomeric counterpart, nudicaulin **VII**, except for some minor shift variations (Table 4), which in part may be due to extraction of the data from 2D heteronuclear correlation spectra (HSQC and HMBC), show analogous 1D and 2D NMR spectra to **VIII**, and therefore seem to have the same skeleton and carbohydrate units, the only difference being the configuration of the chiral centre at C-10 (Fig. 6).

The identical acylated sugar moieties of the kaempferol derivative **5** and both nudicaulins **VII** and **VIII** may be due to broad specificities of glucosyl and malonyl transferases involved in the biosynthesis of the glycosides from the respective aglycones. These properties are probably responsible for the identity of conjugation reactions in the other kaempferol derivatives and nudicaulins. The aglycones of flavonols as well as nudicaulins may be formed from a common C_6–C_3–C_6 precursor, e.g. naringenin chalcone, the nudicaulins by a Diels-Alder or ionic (4+2)-cycloaddition reaction of indole (or an equivalent) to a chalcone-derived compound as presented in Fig. 7. The occurrence of natural products derived from enzymatic (4+2)-cycloaddition in plants and microorganisms and the involvement of so-called Diels-Alderases in their biosynthesis has been a matter of debate since many years (Nomura et al., 1995; Pohnert, 2001; Oikawa, 2005). Recently, the first structure of a Diels-Alderase has been characterised from the phytopathogenic fungus *Macrophoma commelinae* Togashi (Ose et al., 2003), providing insight in the complex mechanism of a Diels-Alder reaction taking place in vivo. The (4+2)-cycloaddition, whether it proceeds through a concerted or ionic mechanism, would fuse the chalcone-derived component with the indole moiety resulting in a putative intermediate, which easily could undergo dehydrogenation by loss of H-2 (α to nitrogen) and benzylic H-11 to form the double bond between carbon atoms C-2 and C-11. Nucleophilic attack of the nitrogen to C-19, which is activated by the enolic structure of the *p*-hydroxyphenyl vinyl residue, finally would close ring **e** (Figs. 6 and 7). However, no experiments were undertaken so far to support this hypothesis.

2.3. Final considerations

In summary, the present study describes the identification of the major flavonoids from petals of the yellow flowering *P. nudicaule* as kaempferol 3-*O*-β-sophoroside, kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside and their mono- and dimalonates and proposes structures for the nudicaulins. Absorption data indicated that the latter are responsible for the intense petal pigmentation. The nudicaulins, having the same acylated glycoside moieties as the kaempferol derivatives, were shown to occur as pairs of optical isomers and one of these pairs, compounds **VII** and **VIII,** was studied in detail by MS, NMR and chiroptical methods, pointing to a novel pentacyclic indole alkaloid skeleton of the aglycones. Further nudicaulins (**III**–**VI**) were characterised as isomeric monomalonyl derivatives by hydrolysis and mass spectrometric analyses, while **I** and **II** are the non-malonylated precursors. It is striking that identical acylated sophorose is attached not only to kaempferol and the nudicaulin aglycones, but also to pelargonidin (Cornuz et al., 1981) in different types of *P. nudicaule*. All the nudicaulins completely differ in their structure from other alkaloids in *P. nudicaule* belonging mainly to the promorphinan and protopine type (Preininger, 1986). The nudicaulins represent a further group of coloured alkaloids (Harborne, 1965) and their accumulation in petals could enhance the attraction of flowers for insects. Concerning the occurrence of nudicaulins in other plants than *P. nudicaule* it is known that Price et al. (1939) found nudicaulin also in yellow flowers of *Meconopsis cambrica* (L.) Vig. This could be confirmed by chromatographic comparison and UV/Vis spectroscopy (Böhm, H., 1984, unpublished) of nudicaulin from both species.

3. Experimental

3.1. Plant material

Seed specimens designated as *P. nudicaule* were obtained from Botanical Gardens and gave rise to fully developed plants. Dark yellow flowering types were propagated and their descendants cultivated in open ground. Flowers were collected daily and the isolated petals stored at −20 °C for lyophilisation. A voucher specimen is deposited in the IPB (WS007/2000).

Table 4
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of isomeric 19-(4-hydroxy- phenyl)-10*H*-1,10-ethenochromeno[2,3-*b*]indole-6,8,18- triol 6-*O*-β-(6-*O*-malonyl)glucoside)-18-*O*-β-[(6-*O*-malonyl)sophoroside], nudicaulin **VII** and **VIII**

No.	**VII**		**VIII**	
	δ, mult., *J*(Hz)	*δ*[a]	*δ*, mult., *J*(Hz)	*δ*[a]
Aglycone				
2		179.8		179.8
4		154.9		154.7
5	6.11, *d*, 2.0	98.0	6.12, *d*, 2.0	97.9
6		159.2		159.3
7	6.06, *d*, 2.0	89.6	6.06, *d*, 2.0	90.2
8		159.4		159.3
9		103.6		103.5
10	5.29, *s*	47.0	5.09, *s*	48.1
11		136.5		136.4
12		124.9		124.6
13	7.91, *d*, 7.7	123.4	7.97, *d*, 7.7	123.9
14	7.20, *dd*, 7.7, 7.7	124.4	7.21, *dd*, 7.7, 7.7	124.7
15	7.39, *dd*, 7.7, 7.7	129.9	7.38, *dd*, 7.7, 7.7	130.1
16	7.52, *d*, 7.7	120.3	7.50, *d*, 7.7	120.5
17		162.7		162.5
18		125.6		126.1
19		150.5		148.9
1′		121.1		121.2
2′/6′	8.16, *d*-like, 8.6	133.7	8.16, *d*-like, 8.6	133.9
3′/5′	6.93, *d*-like, 8.6	115.9	6.99, *d*-like, 8.6	116.1
4′		160.8		160.6
Glc A[b]				
1″	4.80, *d*, 7.7	95.9	4.59, *d*, 7.3	97.3
2″	3.71[a]	76.6	3.38[a]	81.8
3″	3.51[a]	77.2	3.37[a]	76.0
4″	3.13[a]	69.8	3.04[a]	70.1
5″	3.26[a]	74.2	3.28, *m*	74.3
6″A	4.03, *dd*, 5.6, 10.6	63.9	4.17, *dd*, 5.6, 10.6	64.1
6″B	4.22, *d*, 10.6		4.26, *d*, 10.6	
mal-1″[c]		166.9		166.8
mal-2″A	3.16[a]	41.5	3.38[a]	41.5
mal-2″B	3.51[a]		3.42[a]	
mal-3″		167.9		167.9
Glc B				
1‴	4.88, *d*, 8.0	101.6	4.38, *d*, 8.0	104.5
2‴	~3.15[a]	~74	3.02[a]	75.0
3‴	~3.23[a]	~77	3.19[a]	76.4
4‴	~3.07[a]	~70	3.15[a]	69.3
5‴	3.09[a]	77.5	2.88, *m*	76.8
6‴A	3.23[a]	61.6	3.10[a]	61.3
6‴B	3.64[a]		3.20[a]	
Glc C				
1⁗	4.74, *d*, 7.6	100.2	4.71, *d*, 7.7	100.6
2⁗	~3.14[a]	~74	3.15[a]	73.5
3⁗	~3.07[a]	~77	3.07[a]	76.4
4⁗	~3.25[a]	~70	3.15[a]	69.3
5⁗	3.53[a]	73.5	3.52, *m*	73.8
6⁗A	4.07, *dd*, 4.8, 10.8	63.9	4.13, *dd*, 4.8, 10.8	64.1
6⁗B	4.23, *d*, 10.8		4.28, *d*, 10.8	
mal-1⁗		167.1		166.8
mal-2⁗A	3.16[a]	41.5	3.38[a]	41.5
mal-2⁗B	3.51[a]		3.42[a]	
mal-3⁗		168.1		167.9

Compound **VII**: Signals of exchangeable carboxyl protons of the malonate moieties (DMSO-d_6): *δ* 12.7 (*br*). Signals of exchangeable phenolic protons: *δ* ~ 11.9 (*brs*, 8-OH); 10.44 (*s*, 4′-OH).

[a] Chemical shift obtained from HSQC and HMBC spectra.

[b] Glc, glucosyl.

[c] mal, malonyl.

3.2. Isolation and purification of pigments

Lyophilised petals (7.4 g) of *P. nudicaule* were homogenised in 50 ml MeOH/H_2O (9:1) with an Ultra Turrax (Janke & Kunkel, Staufen, Germany) and extracted with stirring overnight at 8 °C. After separation of the extract by reduced pressure filtration the residue was re-extracted twice with 20 ml of the same solvent mixture. The combined extracts were evaporated (3.6 g), dissolved in 50 ml H_2O and partitioned thrice against 100 ml *n*-hexane. The aqueous phase was taken to dryness and re-dissolved in 20 ml H_2O. This crude extract was used for pigment isolation by prep. HPLC.

3.3. HPLC

Analytical and semi-prep. HPLC was performed with a system from Waters (Milford, MA, USA), including the separation module 2690. The liquid chromatograph was equipped with a 5 μm Nucleosil C_{18} column (250 × 4 mm i.d.; Macherey-Nagel, Düren, Germany). The following solvent and gradient systems were used. Solvent system 1: A, 1% aq. HOAc; B, acetonitrile; isocratic 87% A/13% B for 60 min; solvent system 2: A, 1% aq. HOAc; B, acetonitrile; isocratic 82% A/18% B for 60 min; solvent system 3: A, 1.5% aq. H_3PO_4; B, acetonitrile; isocratic 83% A/17% B for 40 min; solvent system 4: A, 1.5% aq. H_3PO_4; B, acetonitrile; constant gradient from 0% B to 60% B in (A+B) within 40 min; the flow rate was 1 ml min^{-1}. The pigments were detected at 350 and 460 nm (injection volume: 5–20 μl).

For prep. HPLC the liquid chromatograph (System Gold; Beckman Instruments, München, Germany) was equipped with a 10 μm-Nucleosil 100–10 C_{18} column (VarioPrep; 250 × 40 mm i.d.; Macherey-Nagel, Düren, Germany). The separation of the flavonoids and nudicaulins was performed with a constant gradient from 65% solvent A (aq. 0.6% HOAc) to 20% solvent A in solvent B (MeOH) within 200 min at a flow rate of 10 ml min^{-1} (injection volume: 2 ml; detection at 350 and 460 nm). **1**: R_t 36–38 min; **2**: R_t 38.5–41 min (3.8 mg); **3**: R_t 45–47 min; **4**: R_t 50–52 min; **5**: R_t 53–57 min (28.8 mg); **6**: R_t 82–85 min (5.7 mg); **7**: R_t 89–93 min (0.6 mg); **8**: R_t 95–99 min; **VII**: R_t 114–120 min (46.7 mg); **VIII**: R_t 124–131 min (36.3 mg). For further purification the fractions were concentrated and separated by chromatography with the same solvent system and the other compounds were purified by semi-prep. HPLC on an analytical column.

3.4. Mass spectrometry

Positive ion electrospray ionisation mass spectra were recorded on a Finnigan MAT TSQ 7000 instrument (electrospray voltage 4.5 kV; capillary 220 °C; sheath gas N_2) coupled to a Micro-Tech Ultra-Plus Micro LC system equipped with a 4 μm C_{18} column (100 × 1 mm i.d, ULTRASEP). For LC, a gradient system starting from 10% B (0.2% aq. AcOH in acetonitrile) in 90% A (0.2% aq. AcOH) to 50% B in (A+B) within 10 min was used, followed by 10 min isocratic elution at a flow rate of 70 μl min^{-1} (injection volume: 2 μl). The CID mass spectra during the LC

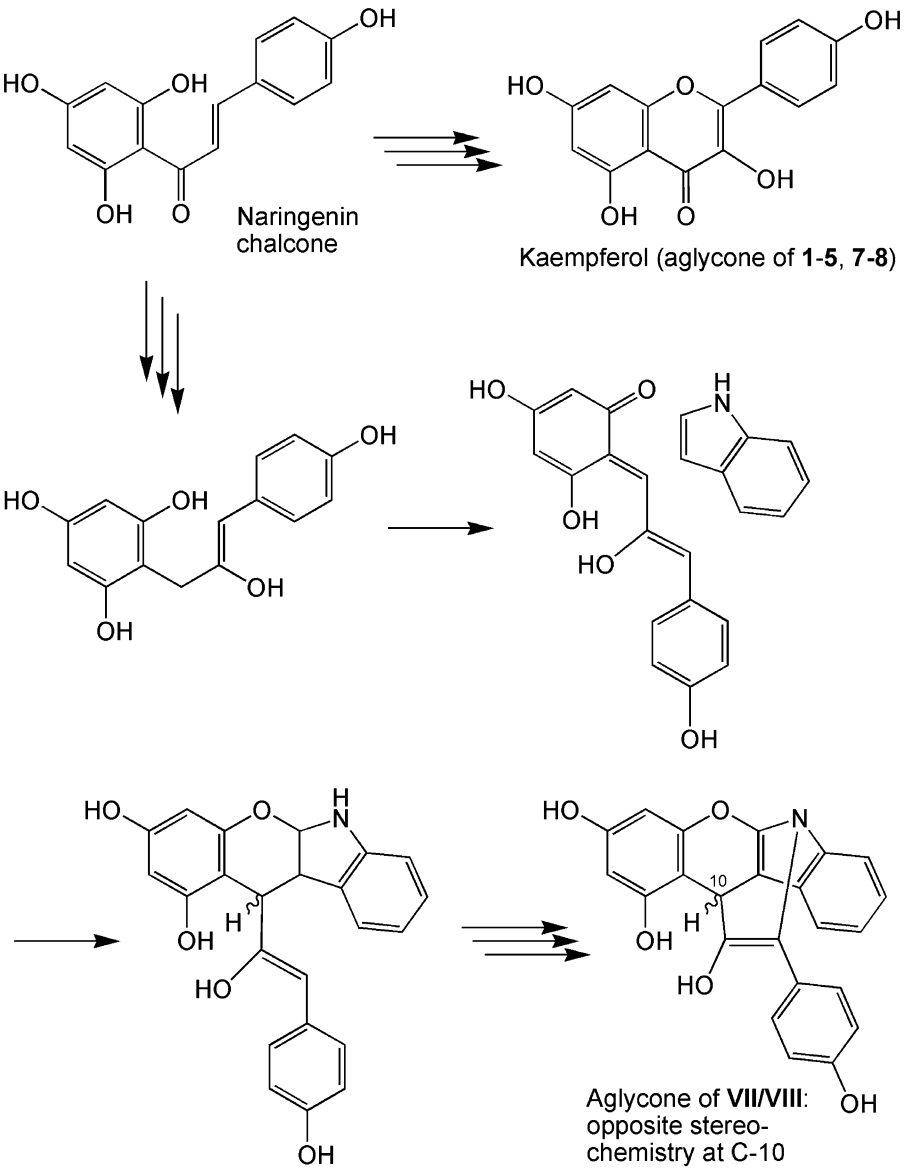


Fig. 7. Hypothetical biosynthesis scheme of nudicaulins **VII** and **VIII** by a Diels-Alder or ionic (4+2)-cycloaddition reaction of a chalcone-derived precursor and indole (or equivalent).

run were obtained under the following conditions: collision energy (collision cell) −25 eV; collision gas: argon; collision pressure: 1.8 mT. All mass spectra are averaged and background subtracted.

The high resolution ESI mass spectra were obtained on a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent off axis spray, voltages: endplate, −3.700 V; capillary, −4.400 V; capillary exit, 100 V; skimmer 1, 15.0 V; skimmer 2, 10.0 V). Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μl h^{-1}. All data were acquired with 512 k data points and zero filled to 2048 k by averaging 32 scans. For producing fragment ions the capillary exit voltage was set to 250 V. ESI-MS data were also recorded on a QTOF2 mass spectrometer (MicroMass, Manchester, UK). The respective sample was applied to a nanospray gold-coated glass capillary placed orthogonally in front of the entrance hole of the instrument. Approximately 1000 V was applied to the capillary, and ions were separated by the time-of-flight analyser. For MS/MS analyses parent ions were selected by the quadrupole mass filter and subjected to collision-induced dissociation using Argon gas. Resulting daughter ions were then separated by the TOF analyser.

Sugars were identified using a standard micro-methylation technique and analyses were performed on a Finnigan GCQ GC-MS as described previously (Nimtz et al., 1996).

3.5. NMR

^{1}H (1D and 2D COSY, 2D HMQC, HMBC, TOCSY, and ROESY) and ^{13}C (1D) NMR spectra were recorded at 300 K on Bruker AVANCE DMX 600, DRX 500, AMX 400 or DPX 300 NMR spectrometers locked to the major signal of the solvents (CD_3OD, DMSO-d_6). Chemical shifts are reported in ppm relative to TMS and coupling constants in Hz.

3.6. Optical measurement

UV/Vis spectra (200–600 nm) (compound **VII** and**VIII**) were recorded in MeOH/H_2O (1:1) and MeOH/H_2O/AcOH (49.5:49.5:1) on a Jasco V-560 UV/Vis spectrophotometer. Optical rotation was measured with a Jasco DIP-1000 digital polarimeter. The CD data (200–500 nm) were recorded on a Jasco J710 spectropolarimeter.

3.7. Hydrolysis experiments

Aq. solutions of **VII** and **VIII** (40 μl) were treated with 40 μl 4 N HCl for 30 min at 100 °C and analysed by HPLC (solvent system 4). For removal of the malonyl moieties aq. solution of **VII** and **VIII** (50 μl) were mixed in an HPLC vial with 20 μl conc. NH_4OH and 30 μl H_2O. The progress of the reaction was monitored by HPLC (solvent system 1).

3.8. Spectral and optical data of petal compounds

Kaempferol 3-*O*-β-sophoroside-7-*O*-β-glucoside (**1**): HR-MS *m/z* 795.1966 ([M+Na]$^+$, $C_{33}H_{40}O_{21}Na$, calc. 795.1954). ESI-MS/MS of *m/z* 773: 773 [M+H]$^+$, 611 [(M+H)-Glc]$^+$, 449 [(M+H)-2 Glc]$^+$, 287 [kaempferol+H]$^+$. Negative ion ESI-MS *m/z* (rel. int.) 771 [M−H]$^-$ (100), 609 [(M−H)$^-$ (18). ^{1}H NMR (CD_3OD): δ 8.11 (2H, *d*-like, $J_{2'/6',3'/5'} = 8.9$ Hz, H-2′/6′), 6.96 (2H, *d*-like, $J_{3'/5',2'/6'} = 8.9$ Hz, H-3′/5′), 6.82 (1H, *d*, $J_{8,6} = 2.1$ Hz, H-8), 6.54 (1H, *d*, $J_{6,8} = 2.1$ Hz, H-6), 5.54 (1H, *d*, $J_{1'',2''} = 7.4$ Hz, H-1″), 5.10 (1H, *d*, $J_{1''',2'''} = 7.4$ Hz, H-1‴), 4.79 (1H, *d*, $J_{1''',2'''} = 7.4$ Hz, H-1‴), 3.78 (1H, *dd*, H-2″), 3.43 (2H, *m*, H-2‴/H-3‴), 3.40 (1H, *dd*, H-2‴), 4.0–3.2 (rest sugar protons, including 3.96 (*dd*, $J_{5,6A} = 2.2$ Hz, $J_{6A,6B} = 12.1$ Hz, H-6A).

Kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-glucoside (**2**): HR-MS *m/z* 881.1954 ($C_{36}H_{42}O_{24}Na$, calc. 881.1958). ESI-MS *m/z* (rel. int.) 881 [M+Na]$^+$ (3), ESI-MS/MS of *m/z* 859: 859 [M+H]$^+$, 697 [(M+H)-Glc]$^+$, 535 [(M+H)-2 Glc]$^+$, 449 [(M+H)-malonyl-2 Glc]$^+$, 287 [kaempferol+H]$^+$. Negative ion ESI-MS *m/z* (rel. int.) 857 [M−H]$^-$ (100), 813 [(M−H)-CO_2]$^-$ (63).^{1}H NMR (CD_3OD): δ 8.07 (2H, *d*-like, $J_{2'/6',3'/5'} = 8.9$ Hz, H-2′/6′), 6.95 (2H, *d*-like,$J_{3'/5',2'/6'} = 8.9$ Hz, H-3′/5′), 6.82 (1H, *d*, $J_{8,6} = 2.1$ Hz, H-8), 6.54 (1H, *d*, $J_{6,8} = 2.1$ Hz, H-6), 5.45 (1H, *d*, $J_{1'',2''} = 7.6$ Hz, H-1″), 5.10 (1H, *d*, $J_{1''',2'''} = 7.5$ Hz, H-1‴), 4.82 (*d*, H-1‴; overlap with residual water signal), 3.80 (1H, *dd*, H-2″), 3.52 (2H, *m*, H-2‴/H-3‴), 3.43 (1H, *dd*, H-2‴). There are three H-6A/H-6B/H-5 systems: 4.26, 4.13, 3.43 (acylated system; $J_{5,6A} = 1.6$ Hz, $J_{5,6B} = 5.0$ Hz, $J_{6A,6B} = 12.0$ Hz), 3.97, 3.75, 3.57 ($J_{5,6A} = 2.2$ Hz, $J_{6A,6B} = 12.0$ Hz), 3.84, 3.74, 3.36 ($J_{5,6A} = 2.4$ Hz, $J_{6A,6B} = 12.0$ Hz), 4.0–3.2 (rest sugar protons).

Compound **3**: ESI-MS *m/z* (rel. int.) 945 [M+H]$^+$ (18), 783 [(M+H)-$^+$ (13), 535 [(M+H)-malonyl-2 Glc]$^+$ (8), 144 (100). Negative ion ESI-MS *m/z* (rel. int.) 943 [M−H]$^-$ (48), 899 [(M−H)-CO_2]$^-$ (58), 855 [(M−H)-2CO_2]$^-$ (100).

Compound **4**: HR-ESI-MS *m/z* 881.1952 ([M+Na]$^+$, $C_{36}H_{42}O_{24}Na$, calc. 881.1958). ESI-MS *m/z* (rel. int.) 859 [M+H]$^+$ (20), 697 [(M+H)-$^+$ (12), 535 [(M+H)-2Glc]$^+$ (100), 287 [kaempferol+H]$^+$ (2). ESI-MS/MS of *m/z* 859: 859 [M+H]$^+$, 697 [(M+H)-$^+$, 535 [(M+H)-2Glc]$^+$, 287 [kaempferol+H]$^+$. Negative ion ESI-MS *m/z* (rel. int.) 857 [M−H]$^-$ (96), 813 [(M−H)-CO_2]$^-$ (100), 609 [(M−H)−malonyl-Glc]$^+$ (20).

Kaempferol 3-*O*-β-[(6-malonyl)sophoroside]-7-*O*-β-[(6-malonyl)glucoside] (**5**): HR-ESI-MS *m/z* 967.1943 ([M+Na]$^+$, $C_{39}H_{44}O_{27}Na$, calc. 967.1962). ESI-MS/MS of *m/z* 945: 945 [M+H]$^+$, 783 [(M+H)-$^+$, 535 [(M+H)-malonyl-2 Glc]$^+$, 517 [535-H_2O]$^+$, 287 [kaempferol+H]$^+$. Negative ion ESI-MS *m/z* (rel. int.) 943 [M−H]$^-$ (21), 899 [(M−H)-CO_2]$^-$ (3), 855 [(M−H)−2CO_2]$^-$ (12), 325 (100). Complete ^{1}H and ^{13}C NMR data are shown in Table 2.

Gossypitrin (gossypetin 7-*O*-β-glucoside) (**6**): ESI-MS *m/z* (rel. int.) 503 [M+Na]$^+$ (5), 242 (100). Negative ion ESI-MS *m/z* (rel. int.) 959 [2M−H]$^-$ (10), 479 [M−H]$^-$ (100), 317 [gossypetin-H]$^-$ (8). Complete ^{1}H and ^{13}C NMR data are shown in Table 3.

Kaempferol 3-*O*-β-sophoroside (**7**): ESI-MS *m/z* (rel. int.) 633 [M+Na]$^+$ (73), 242 (100). Negative ion ESI-MS *m/z* (rel. int.) 609 [M-H]$^-$ (100).^{1}H NMR (CD_3OD): δ 8.08 (2H, *d*-like, $J_{2'/6',3'/5'} = 9.0$ Hz, H-2′/6′), 6.95 (2H, *d*-like,$J_{3'/5',2'/6'} = 9.0$ Hz, H-3′/5′), 6.44 (1H, *d*, $J_{8,6} = 2.1$ Hz, H-8), 6.24 (1H, *d*, $J_{6,8} = 2.1$ Hz, H-6), 5.49 (1H, *d*, $J_{1'',2''} = 7.5$ Hz, H-1″), 4.79 (1H, *d*, $J_{1''',2'''} = 7.4$ Hz, H-1‴), 3.83 (1H, *dd*, $J_{5''',6'''A} = 2.6$ Hz, $J_{6'''A,6'''B} = 12.0$ Hz, H-6‴A), 3.78 (1H, *dd*, $J_{2''-3''} = 8.8$ Hz, H-2″), 3.73 (1H, *dd*, H-6″A), 3.70 (1H, *dd*, $J_{5''',6'''B} = 5.2$ Hz, H-6‴B), 3.64 (1H, *dd*, $J_{2''',3'''} = 8.9$ Hz, $J_{3''',4'''} = 8.9$ Hz, H-3‴), 3.53 (1H, *dd*, $J_{5'',6''B} = 5.4$ Hz, H-6″B), 3.45–3.34 (3H, *m*, H-2‴, H-3‴, H-4‴), 3.40 (1H, *m*, H-4″), 3.33 (1H, *m*, H-5‴), 3.24 (1H, *ddd*, $J_{5'',6''A} = 2.2$ Hz, $J_{4''-5''} = 9.6$ Hz, H-5″).

Compound **8**: HR-ESI-MS *m/z* 633.1422 ([M+Na]$^+$, $C_{27}H_{30}O_{26}Na$, calc. 633.1426). ESI-MS *m/z* (rel. int.) 633 [M+Na]$^+$ (49), 242 (100). Negative ion ESI-MS *m/z* (rel. int.) 609 [M−H]$^-$ (100).

Compound **I**: HR-ESI-MS *m/z* 872.2594 ([M+H]$^+$, $C_{41}H_{46}NO_{20}$, calc. 872.2608).

Compound **II**: ESI-MS *m/z* (rel. int.) 872 [M+H]$^+$ (100), 548 [(M+H)-2Glc]$^+$ (18), 386 [(M+H)−3 Glc]$^+$ (20).

Compound **III**: QTOF ESI-MS/MS of *m/z* 958: 958 [M+H]$^+$, 914 [(M+H)-CO_2]$^+$, 796 [(M+H)$^+$, 548 [(M+H)−malonyl-2 Glc]$^+$, 386 [(M+H)−malonyl-3 Glc]$^+$.

Compound **IV**: QTOF ESI-MS/MS of *m/z* 958: 958 $[M+H]^+$, 914 $[(M+H)\text{-}CO_2]^+$, 796 $[(M+H)\text{-Glc}]^+$, 548 $[(M+H)\text{-malonyl-2 Glc}]^+$, 386 $[(M+H)\text{-malonyl-3 Glc}]^+$.

Compound **V**: HR-MS *m/z* 958.2588 ($[M+H]^+$, $C_{44}H_{48}NO_{23}$, calc. 958.2612). ESI-MS/MS of *m/z* 958: 958 $[M+H]^+$, 634 $[(M+H)\text{-2 Glc}]^+$, 590 $[(M+H)-2\text{Glc-}CO_2]^+$, 386 $[(M+H)\text{-malonyl-3 Glc}]^+$.

Compound **VI**: ESI-MS *m/z* (rel. int.) 958 $[M+H]^+$ (100), 634 $[(M+H)\text{-2 Glc}]^+$ (9).

Compound **VII**: $[\alpha]_D^{26} - 354°$ ($MeOH/H_2O$ 1:1; c 0.0020); CD ($MeOH/H_2O$ 1:1): $[\Theta]_{M\,207} - 88{,}317$, $[\Theta]_{M\,238} - 5971$, $[\Theta]_{M\,272} - 33{,}757$, $[\Theta]_{M\,312} + 366$, $[\Theta]_{M\,379} - 13{,}675$ deg $cm^2\,dmol^{-1}$; $\lambda_{max}^{MeOH/H_2O(1:1)}nm(\log\varepsilon)$: 254 (4.17), 384 (3.96), 470 (3.68), 509 (3.52); $\lambda_{max}^{MeOH/H_2O/AcOH(49.5:49.5:1)}nm$ ($\log\varepsilon$): 224 (4.40), 256 (4.32), 338 (3.80), 461 (4.45). HR-ESI-MS *m/z* 1044.2555 ($[M+H]^+$, $C_{47}H_{50}NO_{26}$, calc. 1044.2616). ESI-MS/MS of *m/z* 1044: 1044 $[M+H]^+$, 1000 $[(M+H)\text{-}CO_2]^+$, 958 $[(M+H)\text{-malonyl}]^+$, 882 $[(M+H)\text{-Glc}]^+$, 634 $[(M+H)\text{-malonyl-2 Glc}]^+$, 590 $[(M+H)\text{-malonyl-2 Glc-}CO_2]^+$, 386 $[(M+H)\text{-2malonyl-3 Glc}]^+$. ^1H and ^{13}C NMR data are shown in Table 4.

Compound **VIII**: $[\alpha]_D^{26} + 370°$ ($MeOH/H_2O$ 1:1; c 0.0021); CD ($MeOH/H_2O$ 1:1): $[\Theta]_{M\,209} + 47{,}011$, $[\Theta]_{M\,238} - 5227$, $[\Theta]_{M\,270} + 35{,}852$, $[\Theta]_{M\,314} - 400$, $[\Theta]_{M\,391} + 17{,}358$ deg $cm^2\,dmol^{-1}$; $\lambda_{max}^{MeOH/H_2O(1:1)}nm(\log\varepsilon)$: 250 (4.06), 386 (3.90), 470 (3.64), 508 (3.45); $\lambda_{max}^{MeOH/H_2O/AcOH(49.5:49.5:1)}nm(\log\varepsilon)$: 228 (4.28), 256 (4.32), 337 (3.81), 464 (4.47). HR-ESI-MS *m/z* 1044.2593 (($[M+H]^+$, $C_{47}H_{50}NO_{26}$, calc. 1044.2616) ESI-MS/MS of *m/z* 1044: 1044 $[M+H]^+$, 1000 $[(M+H)\text{-}CO_2]^+$, 882 $[(M+H)\text{-}^+$, 634 $[(M+H)\text{-malonyl-2 Glc}]^+$, 590 $[(M+H)\text{-malonyl-2 Glc-}CO_2]^+$, 386 $[(M+H)\text{-2 malonyl-3 Glc}]^+$. ^1H and ^{13}C NMR data are shown in Table 4.

Compound **A**: HR-ESI-MS *m/z* 710.2080 (($[M+H]^+$, $C_{35}H_{15}NO_{26}$, calc. 710.2080).

Compound **A***: HR-ESI-MS *m/z* 710.2081 (($[M+H]^+$, $C_{35}H_{15}NO_{26}$, calc. 710.2080) ESI-MS *m/z* (rel. int.) 710 $[M+H]^+$ (100), 386 $[(M+H)\text{-2 Glc}]^+$ (12).

Compound **TP**: HR-ESI-MS *m/z* 1044.2592 (($[M+H]^+$, $C_{47}H_{50}NO_{26}$, calc. 1044.2616).

Acknowledgements

The authors are indebted to B. Kolbe, C. Kuhnt and M. Süße [Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany] and C. Kakoschke, B. Jaschok-Kentner and C. Hanko (German Research Centre for Biotechnology, Braunschweig, Germany) for skilful experimental assistance. Furthermore, we are grateful to C. Kaufmann and A. Kohlberg (IPB) for the figures and photographs. H. Böhm thankfully remembers the cooperation of S. Huneck and G. Schneider (IPB Halle), J. Hempel (German Institute of Human Nutrition Potsdam-Rehbrücke) and J.W. Metzger (University Stuttgart) in foregoing approaches to the nudicaulin structure.

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